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Publication Title:

MODULATION OF IgG BINDING TO FcRn

Abstract:

Abstract of WO9823289

Disclosed are mutant IgG molecules having altered amino acid sequences in the FcRn-binding region. These changes confere increased or decreased affinity for FcRn and thus, respectively, a decreased or increased rate of clearance from the systemic circulation. Such molecules can be attached to detectable labels or cytotoxic moieties for imaging tissues or for delivering cytotoxins. Also disclosed is a method for identifying IgG molecules with altered half-lives in circulation by contacting the molecules with FcRn. Data supplied from the esp@cenet database - Worldwide

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(54) Title: MODULATION OF IgG BINDING TO FcRn

(57) Abstract

Disclosed are mutant IgG molecules having altered amino acid sequences in the FcRn–binding region. These changes confere increased or decreased affinity for FcRn and thus, respectively, a decreased or increased rate of clearance from the systemic circulation. Such molecules can be attached to detectable labels or cytotoxic moieties for imaging tissues or for delivering cytotoxins. Also disclosed is a method for identifying IgG molecules with altered half–lives in circulation by contacting the molecules with FcRn.

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MODULATION OF IgG BINDING TO FcRn
Background of the Invention
The field of the invention is immunoglobulins.
5   Immunoglobulin G (IgG) is used intravenously to treat a number of diseases that involve immune deficiencies, including acquired immune deficiency syndrome (AIDS), idiopathic thrombocytopenic purpura (ITP), Kawasaki disease, Guillaine-Barre Syndrome, and
10 dermatomyositis. Recently, there has also been increasing use in immunosuppression in transplanted patients, and in specifically directed antibody therapy, such as monoclonal antibodies used as a form of cancer chemotherapy.
15   Previously, immunoglobulins were extracted from pooled whole blood, but the increasing risk of infection from AIDS and the related decrease in the available blood supply precipitated increased reliance on murine monoclonal antibodies for in vivo human therapy.
20 Initially, antibodies from such sources had problems with short half-life and inciting an immune response against the murine proteins (the HAMA response), but both of these problems have been alleviated somewhat by the practice of humanizing the antibodies by combining the
25 human constant region with the mouse variable region.
           Although monoclonal antibodies now have a half-life similar to that of native human IgG, there are still situations where it would be desirable to have even more control over the length of time before immunoglobulins
30 are catabolized.
           This is especially true in treating immune deficiency conditions. When the concentration of IgG is increased above normal levels in the circulation, its half-life decreases. Therefore it is difficult to
35 maintain higher-than-normal levels of the immunoglobulins during treatment, and patients require frequent

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injections of the antibodies. On the other hand, in
situations where the antibodies are being used as a means
to target a chemotherapeutic agent, such as a
radionuclide or a protein toxin, to a particular tissue
5 or cell type, the dosage is often limited by the risk of
damage to the bone marrow and other normal tissues due to
non-specific binding. In such cases, a shorter-than-
normal half-life would be desirable.

Summary of the Invention

10   The invention is based on the discovery that FcRn,
in binding to IgG, sequesters it and protects it from
degradation. FcRn is a receptor found on the intestinal
surface of the neonate, and is responsible for the
shuttling of maternal milk IgG from the intestinal lumen
15 through the intestinal epithelial cell into the systemic
circulation. It is now known also to be responsible for
preventing IgG from being cleared from the animal's
circulation. Using the guidance provided herein, one can
create, using recombinant methods, an IgG molecule that
20 has one or more amino acid additions, deletions, or
substitutions (conservative or nonconservative) in the
region that binds to FcRn, thereby either increasing or
decreasing the molecule's affinity for FcRn. An increase
in affinity would translate into the altered IgG's having
25 a longer half-life *in vivo* than native IgG, while a
decrease in affinity for FcRn would have the opposite
effect.

       Although monoclonal antibodies now have a half-
life similar to that of native human IgG, there are still
30 situations where it would be desirable to have even more
control over the length of time before immunoglobulins
are catabolized.

       This is especially true in treating immune
deficiency conditions. When the concentration of IgG is

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increased above normal levels in the circulation, its
half-life decreases. Therefore it is difficult to
maintain higher-than-normal levels of the immunoglobulins
during treatment, and patients require frequent
5 injections of the antibodies. On the other hand, in
situations where the antibodies are being used as a means
to target a chemotherapeutic agent, such as a
radionuclide or a protein toxin, to a particular tissue
or cell type, the dosage is often limited by the risk of
10 damage to the bone marrow and other normal tissues due to
non-specific binding. In such cases, a shorter-than-
normal half-life would be desirable.

An IgG with an increased half-life *in vivo* would
be useful in treating conditions such as acquired immune
15 deficiency syndrome (AIDS) or idiopathic thrombocytopenic
purpura (ITP), where maintaining a higher-than-normal
concentration of circulating IgG is desirable. A mutant
IgG molecule that binds less strongly to FcRn and is
therefore cleared more rapidly would be of benefit where
20 the IgG is being used for chemotherapy or as a tumor
imaging agent.

The mutated IgG of the invention would have amino
acid substitutions in the FcRn-binding region only,
resulting in altered half-life only, with no substantial
25 change in overall immune function.

Also claimed is a method of removing IgG from the
blood of an animal by administration of soluble FcRn,
which would complex with the circulating antibody and
prevent it from being sequestered by cellular FcRn. This
30 method would be especially useful in chemotherapies, to
control the ratios of tumor-bound to circulating
antibodies.

Unless otherwise defined, all technical and
scientific terms used herein have the same meaning as
35 commonly understood by one of ordinary skill in the art

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to which this invention belongs. Although methods and
materials similar or equivalent to those described herein
can be used in the practice or testing of the present
invention, the preferred methods and materials are
5 described below. All publications, patent applications,
patents and other references mentioned herein are
incorporated by reference in their entirety. In case of
conflict, the present specification, including
definitions, will control. In addition, the materials,
10 methods, and examples are illustrative only and not
intended to be limiting.

Other features and advantages of the invention
will be apparent from the following detailed description,
and from the claims.

15   Brief Description of the Drawings
Figure 1 is a graph illustrating clearance of
intravenously injected ^{125}I-immunoglobulins in mice with
and without β2-microglobulin. Approximately 1×10^7
counts per minute of labeled IgG was injected into the
20 external jugular vein. Blood was collected at the time
points indicated. Percent initial radioactivity = counts
per minute per milligram blood at indicated time point x
100 per counts per minute per milligram blood at $t_0 = 1$
minute after injection. Mouse IgG1 is represented by the
25 solid line, chicken IgY by a dashed line, β2m+/+ by
circles, β2m+/- by triangles, and β2m-/- by diamonds; n =
5 for each group.

Figure 2 is a diagram illustrating partial amino
acid sequences of IgG molecules used in this study (EU
30 numbering). Alternative amino acid residues within an
isotype are shown below the most common sequence.
Sequences are from Kabat et al., 1991, Sequences of
proteins of immunological interest, 5th edn., pg. 683,

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NIH, Bethesda, MD. Predicted contacts with FcRn (from
Burmeister et al., 1994, Nature 372:379) are underlined.
Residues for binding to FcRn (Kim et al., 1994, Eur. J.
Immunol. 24:2429; Raghavan et al., 1995, Biochemistry
5 34:14649) are marked with an asterisk.

Figure 3 is an illustration depicting the proposed
role for FcRn in protecting IgG from degradation. IgG
taken up in the fluid phase binds FcRn at acidic pH in
early endosomes. IgG bound to FcRn is recycled to the
10 plasma membrane and released, whereas unbound proteins
are sorted to lysosomes and degraded.

Detailed Description

The altered IgG's of the invention are most
readily prepared by standard recombinant DNA methods,
15 e.g. site-directed mutagenesis or PCR. The regions of
IgG to be mutated corresponds to amino acids 248 through
257, 308 through 314, and 429 through 436 of IgG. Within
these regions, five particular amino acid residues have
been identified as being important in FcRn binding, but
20 others can be explored as well by using DNA primer-based
site-directed mutagenesis, available commercially as a
kit (Amersham, Arlington Heights, Illinois), and well
known in the art. The Amersham kit can be used according
to the manufacturer's instructions in order to mutate
25 specific residues within the FcRn-binding region.

Preliminary in vitro comparison of the binding of
non-native IgG molecules relative to native IgG is done
by radiolabelling the IgG molecules, incubating them with
cells which express FcRn, washing the cells, and then
30 measuring the amount of radioactivity that remains in
association with the cells. Suitable cells include
endothelial cells, cells (such as human embryonic kidney
cell line 293) transfected with a vector encoding FcRn,

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or intestinal epithelial cells from suckling rats or
mice. Alternatively, one could use a brush border
fraction derived from such intestinal epithelial cells,
prepared in accordance with standard methods (e.g.,
5 Wallace and Rees, 1980, Biochem. J. 188:9).

The mutant IgG antibodies can be tested for *in
vivo* half life by radiolabelling with $Na^{125}I$ (DuPont,
Wilmington, DE) using the Iodogen™ method (Pierce
Biochemical, Rockford, IL). Free iodine is removed by
10 gel filtration on Sephadex G-25 and aggregated
immunoglobulins are removed by gel filtration on Sephadex
G-200. The ^{125}I-immunoglobulin can then be diluted in 10%
normal mouse serum to an injection concentration of 1 x
10^7 cpm / 150 μl. Anesthetized mice can be injected in
15 the jugular vein with 150 μl of the radiolabelled
immunoglobulin, and subsequently bled with capillary
tubes from the retro-orbital sinus at serial time points
following injection. Plasma would be collected by
centrifugation and total radioactivity measured in a
20 gamma counter and expressed as cpm/mg blood. The
percentage radioactivity remaining in the blood after the
last bleed would be calculated relative to the value 1
minute after injection. Protein-bound radioactivity
would be measured by precipitation of the plasma in 10%
25 TCA. The clearance curves for the various radiolabelled
antibodies can then be plotted, revealing which produce
antibodies with the desired characteristics. To test
whether immune function is preserved, immunoprecipitation
or ELISA assays can be used to ensure that the antibody
30 does in fact complex with the antigen of interest.

For administration to human patients, the mutated
immunoglobulins of the invention can be humanized by
methods known in the art, e.g., monoclonal antibodies can
be commercially humanized (Scotgen, Scotland; Oxford
35 Molecular, Palo Alto, CA). The antibodies can then be

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purified using known methods, such as absorption onto
immobilized Protein A or immunoaffinity chromatography.
Following purification, the antibodies of the invention
can be administered to patients in a pharmaceutically
5   acceptable excipient such as physiological saline. The
antibodies of the present invention can be administered
by any standard route including intravenously,
intraperitoneally, intramuscularly, or subcutaneously.
It is expected that the preferred route of administration
10 will be intravenous.

As is well known in the medical arts, dosages for
any one patient depends on many factors, including the
patients general health, sex, size, body surface area,
age, as well as the particular compound to be
15 administered, time and route of administration, and other
drugs being administered concurrently. Determination of
correct dosage for a given application is well within the
abilities of one of ordinary skill in the art of
pharmacology.
20   The invention also includes screening methods for
identifying IgG molecules with altered circulating half-
lives and binding affinities relative to native IgG.

Example 1: Increased clearing in mice that lack β2-
microglobulin.
25 **Animals.**   Mice heterozygous for the β2m gene disruption
(Zijlstra et al., 1990, Nature 344:742) were mated and
allowed to deliver. Tail tissue was taken from the pups
for genomic DNA preparation to determine their β2m
genotypes (Israel. et al., 1995, J. Immunol. 154:6246).
30 The clearance experiments were done at 8 weeks of age.
The mice were given 0.01% NaI in their drinking water one
day prior to injection and throughout the period of
monitoring clearance of the ^{125}I-immunoglobulins.

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Preparation of ^{125}I-immunoglobulin for injection: Mouse
IgG1, IgG2a, IgG2b and IgG3 (Cappel, Durham, N.C.) and
chicken IgY (Cappel) were labeled with $Na^{125}I$ (Dupont,
Wilmington, DE) using the Iodogen™ method (Pierce
5 Biochemical, Rockford, IL). Free iodine was removed by
gel filtration on Sephadex G-25 and aggregated
immunoglobulins were removed by gel filtration on
Sephadex G-200. Precipitation in 10% trichloroacetic
acid (TCA) showed that at least 90% of the ^{125}I in
10 preparations of IgG1, IgG3, and IgY was bound to protein,
and at least 80% of the radiolabel in IgG2a and IgG2b was
bound to protein. The ^{125}I-immunoglobulin was diluted in
10% normal mouse serum to achieve approximately 1×10^7
cpm/150 μl for injection.

15 **Clearance experiments:** Under pentobarbitol anesthesia
(65 μg/g body weight), the external jugular veins of β2m-
/-, β2m+/-, and β2m+/+ mice were exposed and injected
with approximately 150 μl of the ^{125}I-immunoglobulin
diluted in 10% normal mouse serum. The mice were bled
20 with capillary tubes from the retro-orbital sinus at
serial time points following injection, under light
isotharine anesthesia. Plasma was collected by
centrifugation and total radioactivity was measured in a
gamma counter and expressed as cpm/mg blood. After the
25 last bleed, the animals were killed with CO_2. The
percentage radioactivity remaining in blood was
calculated relative to the value 1 min. after injection.
Protein-bound radioactivity was measured by precipitation
of the plasma in 10% TCA. This protocol has been
30 approved by the Institutional Animal Care and Use
Committee at Brandeis University.

Pharmacokinetic data analysis: The data from each animal
were fitted to a double exponential model,

$$C = e^{b-k2t} + e^{a-(k1+k2)t},$$

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by a non-linear least squares method with a
multiplicative error structure. The parameters k1 and k2
were constrained to be positive. The area under the
curve at infinity (AUC∞), mean residence time (MRT),
5 terminal elimination half life ($t_{1/2}$), and the phase I
half life were then calculated from a, b, k1, and k2.
Means and s.e.m.s for these parameters were calculated
for each genotype group. Differences between the groups
were tested with an analysis of variance using Gabriel's
10 procedure to make pairwise comparisons (Gabriel, 1978, J.
Amer. Statistical Assoc. 73:724).
Results: The clearance curves for radiolabeled mouse
IgG1 and chicken IgY are shown in Figure 1, which is a
graph showing the clearance of intravenously injected
15 ^{125}I-labelled immunoglobulins in mice with and without $\beta 2$-
microglobulin. The curves are biphasic, with phase 1
representing equilibration between the intravascular and
extravascular compartments and phase 2 representing the
elimination of the protein from the intravascular space.
20 The pharmacokinetic parameters are shown in Table 1. The
phase 1 half lives did not differ significantly for IgG1
and IgY, or between the three $\beta 2m$ genotypes. However,
there were significant differences in the phase 2
(terminal) half lives ($t_{1/2s}$). Mouse IgG1 was degraded
25 more rapidly in $\beta 2m-/-$ mice than in $\beta 2m+/-$ or $\beta 2m+/+$
littermates. Specifically, the half life was 25 hours
in the $\beta 2m-/-$ animals compared to 5 days for the $\beta 2m+/+$
mice (Fig. 1). There was no significant difference
between the clearance in heterozygous ($t_{1/2}$ = 123 h) and
30 wild type mice, suggesting that the effect on IgG
catabolism was not related to the dose of the $\beta 2m$ gene.
The radioactivity left in the serum was 95-98%
precipitable by TCA at all time points (data not shown),
suggesting the radioactivity measured was indeed bound to
35 IgG. There was little release of TCA-soluble

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radioactivity into the systemic circulation during the time period studied.

Radiolabeled chicken IgY was cleared equally rapidly in wild type mice and in mice homozygous for the disruption in the $\beta 2m$ gene (Figure 1). The terminal half life of IgY (21-22 hours) was not significantly different than that of mouse IgG1 I in $\beta 2m-/-$ mice (Table 1).

Table 1.Pharmacokinetic parameters of $^{125}I\text{-IgG}$ clearance.

$\beta 2m$ genotype (n)	Ig isotype	$AUC\infty^a$ x 10^7	MRT^b (hours)	Phase I half life (hours)	$t_{1/2}^c$ (hours)
+/+ (2)	$mIgG1^d$	35 (8)^f	167 (6)	3.1 (0.1)	120 (3)
+/- (4)	mIgG1	33 (3)	168 (14)	6.7 (1.7)	123 (10)
-/- (4)	mIgG1	8.3 (0.9)	29.7(3.6)	5.5 (0.4)	25 (3.6)
+/+ (3)	$cIgY^e$	3.7 (0.2)	15.7(0.8)	5.4 (0.6)	22 (1.2)
-/- (3)	cIgY	3.8 (0.1)	18.7(3.0)	4.6 (1.2)	21 (0.5)

a = Area under the curve
b = Mean residence time
c = Terminal half life
d = Mouse IgG1
e = Chicken IgY
f = Mean (s.e.m.)

Pharmacokinetic parameters for the clearance of mouse IgG1, IgG2a, IgG2b and IgG3 compared in a separate experiment in $\beta 2m-/-$, +/- and +/+ mice are shown in Table 2. The half lives of IgG1, IgG2a and IgG3 were significantly lower in $\beta 2m-/-$ mice than in $\beta 2m+/-$ or +/+ siblings. The half lives of IgG2b in mice of the three $\beta 2m$ genotypes were not significantly different, although degradation appeared degradation appeared more rapid in $\beta 2m-/-$ mice.

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Table 2. Pharmacokinetic parameters of mouse ^{125}I-IgG subclass clearance.

Ig Isotype	β2m genotype (n)	AUC∞[a] x 10^7	MRT[b] (hours)	Phase I half life (hours)	t$_{1/2}$[c] (hours)
IgG1	+/+, +/1 (3)	10.7(2.0)[d]	114 (38)	2.7 (0.4)	84 (27)
IgG1	-/- (3)	3.3 (0.4)	31 (4)	3.8 (2.2)	25 (1.4)
IgG2a	+/+, +/- (3)	2.1 (0.4)	29 (2)	2.2 (0.3)	33 (1.3)
IgG2a	-/- (3)	1.7 (0.1)	16 (1)	2.0 (0.2)	21 (0.6)
IgG2b	+/+, +/- (3)	1.7 (0.04)	35 (1)	1.6 (0.1)	42 (1.9)
IgG2b	-/- (3)	1.1 (0.2)	12 (1)	2.3 (0.8)	33 (10)
IgG3	+/+, +/- (3)	8.4 (0.5)	97 (9)	2.7 (0.2)	76 (7.0)
IgG3	-/- (2)	2.5 (0.3)	16 (2)	4.4 (1.8)	24 (7.0)

a = Area under the curve
b = Mean residence time
c = Terminal half life
d = Mean (s.e.m.)

Example 2:   Generation and Screening of IgG mutants.

Five amino acid residues (Ile 253, His 310, Gln 311, His 433, and Asn 434) in the conserved portion of IgG have been identified as being important in FcRn binding. These are shown in Figure 2, which is a diagram showing alignment of partial amino acid sequences of immunoglobulin molecules used in Example 1 (EU numbering). Alternative amino acid residues within an isotype are shown below the most common sequence (Kabat et al., 1991, Sequences of proteins of immunological interest, 5th edn., pp. 683, 692, NIH, Bethesda, MD). Predicted contacts (Burmeister et al., 1994, Nature:372:379) with FcRn are underlined. Residues required for the protection of IgG from rapid degradation and for binding to FcRn (Kim et al., 1994, Eur. J. Immunol. 24:2429; Raghavan et al., 1995, Biochemistry 34:14649) are marked with an asterisk. Other amino acids can be identified using known methods.

To create a mutant IgG molecule, a cDNA encoding the expressed IgG Fc-binding fragment is cloned from IgG hybridoma cells. Site-directed mutagenesis is used to

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substitute specific amino acid residues in positions 248
through 257, 308 through 314, and 429 through 436, using
a commercially-available DNA primer-based site-directed
mutagenesis kit (Amersham, Arlington Heights, Illinois),
5 according to the manufacturer's instructions.

The binding affinity of the mutant IgG antibodies
to FcRn can be tested by immobilizing FcRn on a solid
substrate e.g., a Sepharose™ bead, by standard methods.
An anti-IgG monoclonal antibody (of other than IgG
10 isotype) is labelled with ^{125}I (Dupont, Wilmington, DE)
using the Iodogen™ method (Pierce Biochemical, Rockford,
IL). Free iodine is removed by gel filtration on
Sephadex G-200. The immobilized FcRn is contacted with
0.5 μg/ml of the test IgG plus the ^{125}I-labeled antibody
15 (e.g. for 16-18 hours at 37°C), then washed. The amount
of radioactivity remaining associated with the
immobilized FcRn is measured, and the binding affinity
calculated using well known methods. Alternatively,
binding affinity may be evaluated using an ELISA assay
20 known in the art.

Further testing in a cell-based system may also be
carried out. Confluent layers of FcRn-expressing cells
(e.g., cells transfected with a vector encoding FcRn) are
incubated with 0.4 μ/ml ^{125}I-labeled native or mutant IgG
25 to allow IgG-FcRn binding (e.g., overnight 16-18 hours at
37°C), and washed with medium (complete RPMI, 10% FCS;
Gibco, Grand Island, NY). Cells are then detached by
incubation with 5 mM Na_2EDTA in 50 mM phosphate buffer
(pH 7.5) for 5 minutes. The cells are pelleted and
30 resuspended in 2 ml 2.5 mg/ml CHAPS, 0.1 M Tris-HCl
(pH 8.0), 0.3 mg/ml PMSF, 25 μg/ml pepstatin and 0.1
mg/ml aprotinin and incubated for 30 minutes at room
temperature. The suspension is centrifuged at 12,000 x g
for 30 minutes and the amount of radioactivity in pellets
35 and supernatants determined as an indication of the level

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of binding of the test IgG to FcRn. Alternatively, the
test IgG can be unlabelled, and its binding detected with
a labelled anti-IgG antibody as described above.

        Final studies on *in vivo* clearance of the mutant
5 IgG molecules relative to the native IgG are conducted as
described in Example 1.

Example 3: Therapeutic use of IgG with increased or
decreased rate of clearance.

        For administration to human patients, mutant IgG
10 molecules can be humanized by methods known in the art,
e.g, monoclonal antibodies can be commercially humanized
(Scotgene, Scotland; Oxford Molecular, Palo Alto, CA).
Mutant IgG molecules can be purified using known methods,
such as absorption onto immobilized Protein A or
15 immunoaffinity chromatography. Following purification,
the mutant IgG molecules of the invention or
immunologically active fragments thereof can be
administered to patients in a pharmaceutically acceptable
excipient such as physiological saline. The mutant IgG
20 molecules or other compounds of the invention, e.g.,
mutant IgG molecules linked to therapeutic agents such as
cytotoxic moieties (e.g., radionuclides or toxic
polypeptides such as ricin, *Pseudomonas* exotoxin A, and
diphtheria toxin), can be administered by any standard
25 route including intraperitoneally, intramuscularly,
subcutaneously, or intravenously. It is expected that
the preferred route of administration will be
intravenous. These compounds can be administered
systemically to the bloodstream.
30   As is well known in the medical arts, dosages for
any one patient depends on many factors, including the
patients general health, sex, size, body surface area,
age, as well as the particular compound to be
administered, time and route of administration, and other

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drugs being administered concurrently. Dosages for
compounds of the invention will vary, but a preferred
dosage for intravenous administration is approximately 1
μg to 500 μg/ml/blood volume. Determination of correct
5   dosage for a given application is well within the
abilities of one of ordinary skill in the art of
pharmacology. The optimal dosage may be adjusted
according to the condition of the patient and response of
the patient to therapy.

10   Where the IgG carries a cytotoxic moiety, as for
cancer chemotherapy, rapid clearance would be desired and
so mutant IgGs with reduced FcRn binding are chosen. For
therapeutic use of nontoxic IgG molecules, a decreased
clearance rate is desired, and so mutant IgG molecules
15 with increased affinity for FcRn are selected.

Example 4: Diagnostic use of IgG with increased rate of
clearance. Labelled IgG molecules of the invention can
be used diagnostically. In these situations, increased
clearance of IgG is desirable so that nonspecifically
20 bound labelled antibody is quickly removed from the body,
thereby reducing background. Hybridoma strains producing
IgG molecules specific for target tissue, e.g., cancer
cells, are used, and the IgG molecules are mutated as
described in Example 2. Testing for clearance is also
25 conducted as in Example 2. IgG molecules with decreased
binding to FcRn, compared to wild type molecules, are
chosen.

Other Embodiments
        It is to be understood that while the invention
30 has been described in conjunction with the detailed
description thereof, that the foregoing description is
intended to illustrate and not limit the scope of the

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invention, which is defined by the scope of the appended claims.

Other aspects, advantages, and modifications are within the scope of the following claims.

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SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Jacobowitz Israel, Esther
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5   (ii) TITLE OF THE INVENTION: MODULATION OF IgG
BINDING TO FcRn

(iii) NUMBER OF SEQUENCES: 15

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10 (B) STREET: 225 Franklin Street
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15 (v) COMPUTER READABLE FORM:
    (A) MEDIUM TYPE: Diskette
    (B) COMPUTER: IBM Compatible
    (C) OPERATING SYSTEM: DOS
    (D) SOFTWARE: FastSEQ Version 2.0

20 (vi) CURRENT APPLICATION DATA:
    (A) APPLICATION NUMBER: 00786/360002
    (B) FILING DATE:
    (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
25 (A) APPLICATION NUMBER: 60/031,607
    (B) FILING DATE: November 27, 1996

(viii) ATTORNEY/AGENT INFORMATION:
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35   (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 10 amino acids
    (B) TYPE: amino acid

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```
        (C) STRANDEDNESS: N/A
        (D) TOPOLOGY: linear

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

  Lys Asp Val Leu Thr Ile Thr Leu Thr Pro
5  1             5                 10


           (2) INFORMATION FOR SEQ ID NO:2:

       (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 7 amino acids
          (B) TYPE: amino acid
10         (C) STRANDEDNESS: N/A
          (D) TOPOLOGY: linear

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

  Ile Met His Gln Asp Trp Leu
   1             5


15         (2) INFORMATION FOR SEQ ID NO:3:

       (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 8 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: N/A
20         (D) TOPOLOGY: linear

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

  His Glu Gly Leu His Asn His His
   1             5


           (2) INFORMATION FOR SEQ ID NO:4:

25     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 10 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: N/A
          (D) TOPOLOGY: linear

30    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

  Lys Asp Val Leu Met Ile Ser Leu Ser Pro
   1             5                 10


           (2) INFORMATION FOR SEQ ID NO:5:
```

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```
            (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 7 amino acids
               (B) TYPE: amino acid
               (C) STRANDEDNESS: N/A
 5             (D) TOPOLOGY: linear

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

       Ile Gln His Gln Asp Trp Met
        1               5


            (2) INFORMATION FOR SEQ ID NO:6:

10          (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 8 amino acids
               (B) TYPE: amino acid
               (C) STRANDEDNESS: N/A
               (D) TOPOLOGY: linear

15          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

       His Glu Gly Leu His Asn His Leu
        1               5


            (2) INFORMATION FOR SEQ ID NO:7:

            (i) SEQUENCE CHARACTERISTICS:
20             (A) LENGTH: 10 amino acids
               (B) TYPE: amino acid
               (C) STRANDEDNESS: N/A
               (D) TOPOLOGY: linear

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

25     Lys Asp Val Leu Met Ile Ser Leu Thr Pro
        1               5                   10


            (2) INFORMATION FOR SEQ ID NO:8:

            (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 7 amino acids
30             (B) TYPE: amino acid
               (C) STRANDEDNESS: N/A
               (D) TOPOLOGY: linear

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

       Ile Gln His Gln Asp Trp Met
35      1               5
```

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(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 8 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: N/A
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

His Glu Gly Leu Lys Asn Tyr Tyr
1   5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 10 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: N/A
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Lys Asp Ala Leu Met Ile Ser Leu Thr Pro
1   5   10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 7 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: N/A
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ile Gln His Gln Asp Trp Met
1   5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 8 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: N/A
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

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```
His Glu Ala Leu His Asn His His
1               5
```

(2) INFORMATION FOR SEQ ID NO:13:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 10 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS: N/A
      (D) TOPOLOGY: linear

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Pro Gly Glu Leu Tyr Ile Ser Leu Asp Ala
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:14:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 7 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS: N/A
      (D) TOPOLOGY: linear

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Val Ser Thr Gln Asp Trp Leu
1               5
```

(2) INFORMATION FOR SEQ ID NO:15:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 8 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS: N/A
      (D) TOPOLOGY: linear

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
His Glu Ala Leu Pro Met Arg Phe
1               5
```

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What is claimed is:

1.   A non-naturally occurring IgG molecule having
an altered amino acid sequence compared to native IgG,
and which binds to FcRn with increased affinity relative
5 to native IgG, the alteration comprising an addition or
deletion of an amino acid within the FcRn-binding region
of native IgG, or a substitution of an amino acid within
said region.

2.   A non-naturally occurring IgG molecule having
10 an altered amino acid sequence compared to native IgG,
and which binds to FcRn with decreased affinity relative
to native IgG, the alteration comprising an addition or
deletion of an amino acid within the FcRn-binding region
of native IgG, or a substitution of an amino acid within
15 said region.

3.   The IgG molecule of claim 1, in which at
least one of the following amino acids conserved in
native IgG is substituted with another amino acid: Ile
253, His 310, Gln 311, His 433, Asn 434.

20   4.   The IgG molecule of claim 2, in which at
least one of the following amino acids conserved in
native IgG is substituted with another amino acid: Ile
253, His 310, Gln 311, His 433, Asn 434.

5.   A purified preparation of the IgG of claim 1.

25   6.   A purified preparation of the IgG of claim 2.

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7.   A method of therapy comprising identifying an
animal having a condition treatable with IgG and
administering a therapeutically effective amount of the
molecule of claim 1 to the animal.

5   8.   A method of therapy comprising identifying an
animal having a condition treatable with IgG and
administering a therapeutically effective amount of the
molecule of claim 2 to the animal.

9.   The method of claim 7, wherein said condition
10 is selected from the group consisting of idiopathic
thrombocytopenic purpura (ITP), Kawasaki disease,
acquired immunodeficiency syndrome (AIDS), Guillain-Barre
Syndrome, and dermatomyositis.

10.   A composition comprising the molecule of
15 claim 2 complexed to a cytotoxic moiety.

11.   The composition of claim 10, wherein the
cytotoxic moiety is a toxic polypeptide or a
radionuclide.

12.   A composition comprising the molecule of
20 claim 2, wherein the molecule comprises a cytotoxic
radionuclide.

13.   A therapeutic method comprising indentifying
an animal in need of treatment with an immunotoxin, and
administering a therapeutically effective amount of the
25 molecule of claim 10 to the animal.

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14. A method of imaging a particular tissue in an animal, comprising

(a) providing the IgG molecule of claim 2, wherein the IgG molecule binds preferentially to said tissue and is detectably labelled; and

(b) administering the IgG molecule to the animal in an amount effective to permit the tissue to be imaged.

15. A method for identifying an IgG molecule with an altered half-life in circulation relative to native IgG, comprising

(a) contacting a molecule of FcRn with a candidate non-native IgG in vitro, and

(b) determining whether the candidate non-native IgG binds to FcRn with an affinity higher or lower than that of native IgG, wherein an affinity higher than that of native IgG indicates that the candidate non-native IgG has a half life in circulation greater than that of native IgG, and an affinity lower than that of native IgG indicates that the candidate non-native IgG has a half life in circulation shorter than that of native IgG.

16. The method of claim 15, wherein the FcRn is soluble FcRn.

17. The method of claim 15, wherein the FcRn is bound to a cell.

18. A method of increasing the rate of clearance of circulating IgG, comprising

(a) identifying an animal in need of an increased rate of clearance of circulating IgG, and

(b) administering soluble FcRn to the animal in an amount sufficient to increase the rate of clearance of circulating IgG.

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19.   The method of claim 18, comprising administering IgG to the animal concurrently with the soluble FcRn.

20.   The method of claim 18, wherein the circulating IgG comprises IgG complexed with a cytotoxic moiety.

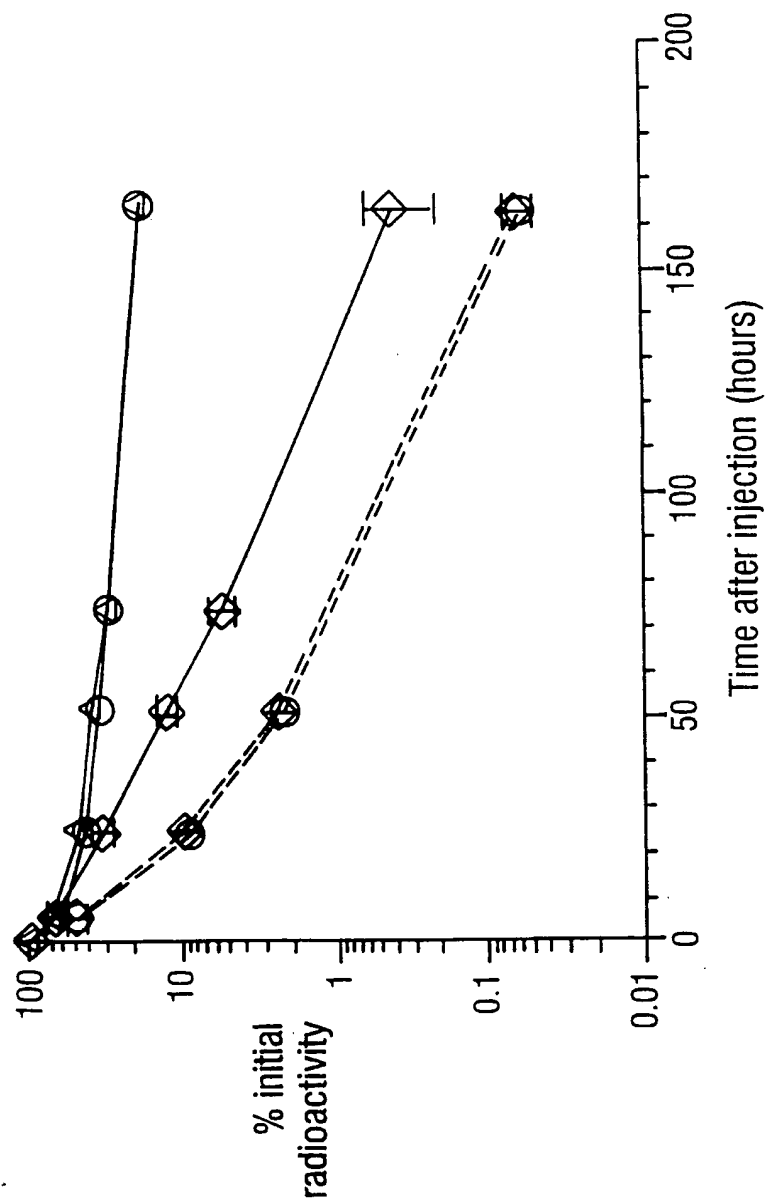21.   The method of claim 18, wherein the circulating IgG is a tumor imaging agent.

1/4

FIG. 1

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```
            248                    *              257
mIgG1    Lys Asp Val Leu Thr Ile Thr Leu Thr Pro     (SEQ ID NO: 1)
                 Thr     Leu     Val


            308    *   *      314
mIgG1    Ile Met His Gln Asp Trp Leu                  (SEQ ID NO: 2)


            429          *   *      436
mIgG1    His Glu Gly Leu His Asn His His              (SEQ ID NO: 3)


            248                             257
mIgG2a   Lys Asp Val Leu Met Ile Ser Leu Ser Pro     (SEQ ID NO: 4)
             Asn


            308              314
mIgG2a   Ile Gln His Gln Asp Trp Met                  (SEQ ID NO: 5)


            429                  436
mIgG2a   His Glu Gly Leu His Asn His Leu              (SEQ ID NO: 6)
                 Val                 His


            248                             257
mIgG2b   Lys Asp Val Leu Met Ile Ser Leu Thr Pro     (SEQ ID NO: 7)
                                         Ser


            308              314
mIgG2b   Ile Gln His Gln Asp Trp Met                  (SEQ ID NO: 8)


            429                  436
mIgG2b   His Glu Gly Leu Lys Asn Tyr Tyr              (SEQ ID NO: 9)


            248                             257
mIgG3    Lys Asp Ala Leu Met Ile Ser Leu Thr Pro     (SEQ ID NO: 10)


            308              314
mIgG3    Ile Gln His Gln Asp Trp Met                  (SEQ ID NO: 11)

            429                  436
mIgG3    His Glu Ala Leu His Asn His His              (SEQ ID NO: 12)
```

FIG. 2A

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```
           248                                    257
cIgY       Pro Gly Glu Leu Tyr Ile Ser Leu Asp Ala    (SEQ ID NO: 13)


           308                    314
cIgY       Val Ser Thr Gln Asp Trp Leu               (SEQ ID NO: 14)


           429                      436
cIgY       His Glu Ala Leu Pro Met Arg Phe            (SEQ ID NO: 15)
```
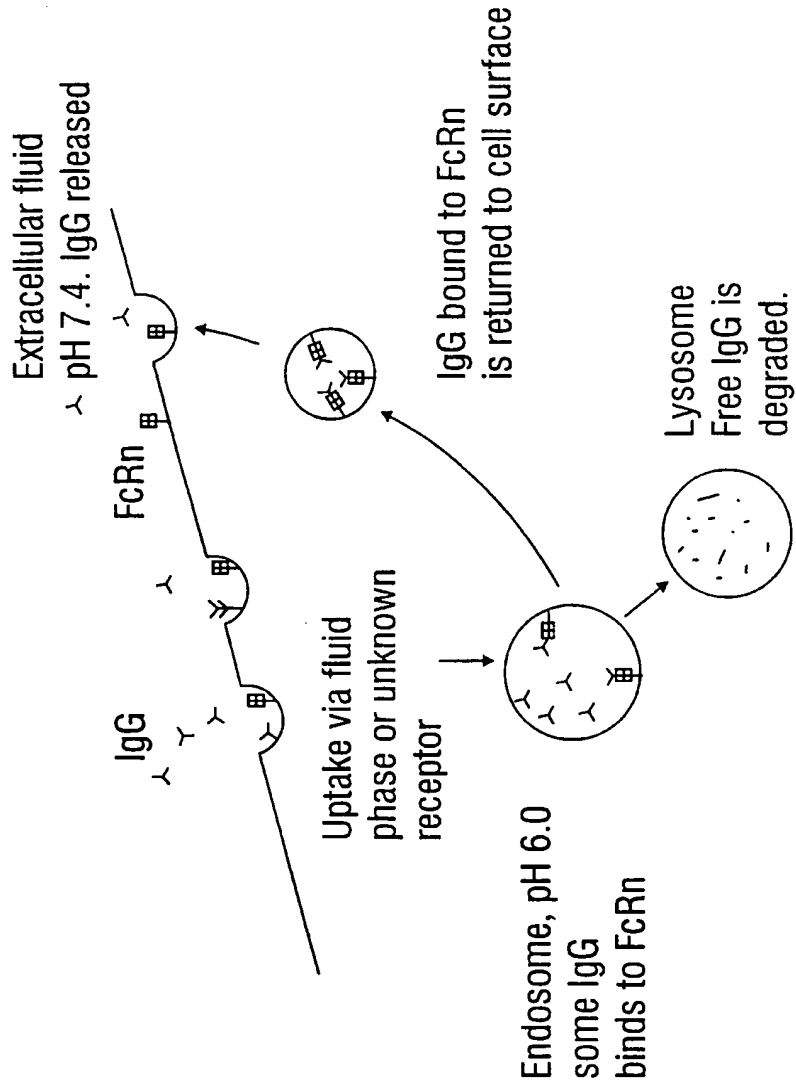
FIG. 2B

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FIG. 3

INTERNATIONAL SEARCH REPORT

A.	CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/395; C07K 16/00
US CL :424/130.1, 133.1, 143.1; 530/387.1, 387.3, 388.1, 388.22
According to International Patent Classification (IPC) or to both national classification and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 133.1, 143.1; 530/387.1, 387.3, 388.1, 388.22

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Examiner's Antibody files.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG (file=medline), key words: antibod? FcRn, neonate

C.	DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	US 5,623,053 A (GASTINEL et al.) 22 April 1997, see entire document.	1-21
X --- Y	RAGHAVAN, M. et al., Analysis of the pH dependence of the neonatal Fc receptor/Immunoglobulin G interaction using antibody and receptor variants, Biochemistry 1995, Vol. 34, pages 14649-14657, see entire document.	1-2 ------- 1-21
Y	SIMISTER, N. E. et al., An Fc receptro structurally-related to MHC Class I antigens, Nature, 12 January 1989, Vol. 337, pages 184-187, see entire document.	1-21

X	Further documents are listed in the continuation of Box C.		See patent family annex.

*	Special categories of cited documents.	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 MARCH 1998	16 APR 1998
Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	STORY, C. M. et al., A major histocompatibility complex Class I-like Fc receptor cloned from human placenta: possible role in transfer of immunoglobulin G from mother to fetus, J. Exp. Med., December, 1994, Vol. 180, pages 2377-2381, see entire document.	1-21